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Electrochemical bromination of organosulfur containing species for the determination of the strength of garlic (*A. sativum*)

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ABSTRACT

The extraction by ethyl acetate and subsequent electrochemical detection of organosulfur containing molecules from garlic is demonstrated. The electrochemical results first evidence the high sensitivity of the process towards the model compound propyl disulfide. Through the in situ formation of bromine at a platinum electrode the propyl disulfide can be readily detected at concentrations as low as 12.5 μM. Second, the work focuses on the detection of organosulfur from fresh garlic samples. Extraction of the organosulfur 'flavour' molecules is achieved with ethyl acetate. Addition of this extract to the electrochemical cell results in an analytically useful signal allowing the voltammetric peak height to be successfully correlated with the garlic strength, as measured using an organoleptic tasting panel.

1. Introduction

One of the most distinctive features of the genus *Allium* is the plants use of organosulfurs as defensive secondary metabolites (Block, 2010). In the cell, sulfoxide precursors are stored within the cytoplasm and the enzyme alliinase is compartmentalised within the vacuole (Lancaster & Collin, 1981). On cell damage, the sulfoxides are converted by the released alliinase to thiosulfinates (organosulfur structures are shown in Fig. 1). The main thiosulfinate formed by garlic (*Allium sativum*) is allicin and this chemical species has been shown to stimulate the nociceptor TRPA1, hence, the perceived pungency of fresh garlic (Macpherson et al., 2005). However, allicin is a reactive species and undergoes a number of different transformations leading to a host of different possible poly- and disulfides (Block, 2010). Consequently, garlic 'flavour' relates to a range of different organosulfurs produced from the precursor sulfoxides. The sulfoxides generally constitute between 2 g kg^{-1} and 25 g kg^{-1} of the fresh weight of the bulb (Hornícková et al., 2010). It is the concentration of these sulfoxides that determine the levels of the thiosulfinates present after preparation of the garlic and hence they ultimately determine the strength' of the garlic in terms of its flavour. However, the concentration of these sulfoxides is found to be significantly influenced by the sub-species, harvest time and storage conditions (Hornicková et al., 2011). For instance the concentration of the sulfoxides has been previously found to increase by ~30% over the first eight weeks of storage (Hornícková et al., 2010). Although numerous methods are available for the quantification of the concentration of the sulfoxides, most importantly HPLC (Ziegler & Sticher, 1989), mass-spectrometry (Lee, Kim, & Lee, 2003) and capillary electrophoresis (Kubec & Dadáková, 2008), none of these methods are easily adaptable for being undertaken by non-scientifically skilled personnel, for the regular determination of batch-to-batch variability.

In the food industry the variation in garlic strength is routinely investigated by organoleptic tasting panels. For these panels macerated garlic is diluted in sour cream (Toebe, Hoojjat, Hernandez, Giacin, & Harte, 1990) and the panel asked to rank the strength of the garlic on a scale of 0–8. Although, this yields some information regarding the strength of the garlic, the procedure is clearly liable to errors due to the subjectivity of the measurement. Previous work by Compton et al. demonstrated how the strength of macerated garlic may be objectively quantified via electrochemical bromination of organosulfur (Martindale, Aldous, Rees, & Compton, 2011). The reactivity of bromine towards organosulfurs has long been recognised, where under non-aqueous conditions the reaction of thiols with bromine is used for the near quantitative formation of the associated disulfide (Wu, Rieke, & Zhu, 1996). However, under aqueous conditions the oxidation can go further, leading ultimately to the sulfonate (Oae & Doi, 1991; Young, 1937). The

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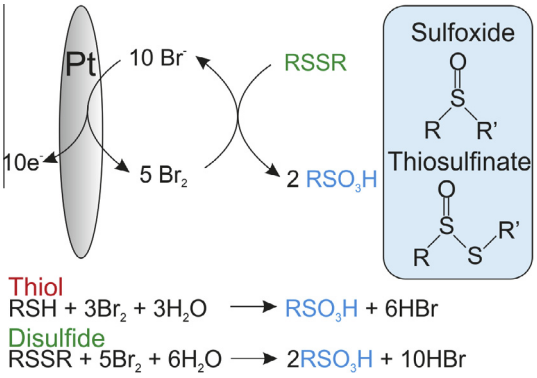

Fig. 1. Schematic showing the electrocatalytic cycle used for the detection of organosulfurs. The bromide/bromine redox system acts as the homogeneous redox mediator, which is oxidized to bromine at the platinum electrode (Pt). Hence, the recorded electrochemical signal is proportional to the organosulfur concentration. The stoichiometry for the reactions shown corresponds to that found in the literature for the complete oxidation of thiol and disulfide species in an aqueous system by bromide (Oae & Doi, 1991). The inset blue box depicts the chemical structures of the flavour pre-cursor sulfoxide and the main 'fresh' flavour molecule thiosulfinate.

equations, presented in Fig. 1, outline the stoichiometry for the reaction of bromine with both thiols and disulfides.

In the electrochemical experiment the organosulfur compounds are extracted from the raw garlic and added to the electrochemical cell. The electrochemical cell contains bromide which is oxidised to bromine at the electrode. This formed bromine reacts with the organosulfur leading to the reformation of the bromide ions. These bromide ions may then be re-oxidised at the electrode surface to bromine. This catalytic cycle (also shown in Fig. 1), which results in the bromide being repeatedly oxidised, leads to an enhancement of the recorded electrochemical current. This increase in the current is directly related to the concentration of the organosulfur compounds present within solution and hence can be directly correlated to the strength of the garlic.

This work builds on the previous paper (Martindale et al., 2011) and through optimisation of the electrochemical conditions in terms of bromide concentration and electrode material, the sensitivity of the technique is enhanced, such that 12.5 μM of the model compound propyl disulfide is experimentally readily detectable. Second, the previous work is hindered by the use of relatively large quantities of acetonitrile (methyl cyanide) as the solvent used for the organosulfur extraction. This works utilises the significantly less toxic solvent ethyl acetate. Finally, having optimised the procedure the electrochemical quantification of the strength of six different garlic samples (comprising of four different sub-species) is evidenced and a strong correlation (R^2 = 0.974) between the electrochemical and independent organoleptic tests is demonstrated, validating the developed methodology for the rapid quantification of the strength of garlic.

2. Experimental

All chemicals were purchased at the highest grade available and used directly without any further purification. Propyl disulfide (98%) and $NaClO_4$ (ACS Reagent, 98%) were sourced from Sigma Aldrich, UK; NaBr was sourced from May and Baker Ltd, UK. All solutions were prepared with ethyl acetate (HPLC Gradient grade, Sigma-Aldhrich >99.7%) and deionised water of resistivity not less than 18.2 MΩ cm at 298 K (Millipore UHQ, Vivendi, UK).

Garlic samples were provided by Beacon Foods Ltd (Brecon Powys, UK), and consisted of 6 samples; Chinese, Fresh White, Red Morado and three samples of Spanish Morado. The garlic cloves were peeled and chopped (macerated) in Beacon Foods Ltd kitchens then posted under refrigerated conditions. Extraction of the organosulfur compounds from the raw macerated garlic samples was achieved by addition of a given mass of garlic (0.1–1.0 g) to 2 mL of ethyl acetate contained in a centrifuge tube. The resulting mixture was subsequently mixed on a Vortex (Whirlimixer, Fisons Scientific Loughbough) for 1 min, and then separated by centrifugation (Centrifuge 5702, Eppendorf, Hamburg, Germany) at 3000 relative centrifugal force (rcf) for 6 min. The resulting ethyl acetate supernatant containing the garlic organosulfur extract was added to the electrochemical cell.

All voltammetric measurements were recorded using an Autolab 101 computer-controlled potentiostat (Metrohm, Utrecht, The Netherlands). Experiments were performed using a three-electrode set-up, with a graphite rod and a Saturated Calomel Electrode (SCE + 0.244 V vs SHE, BASi Inc., Japan) as counter and reference, respectively. An in-house produced platinum macro-electrode (radius: 1.0 mm) was used as the working electrode, the electrode was prepared by securing the material inside an insulating PTFE surround to leave an exposed circular geometric surface. Renewal of the platinum surface was achieved by polishing with alumina slurries (1.0–0.3 μm, Buehler Ltd., USA). A beaker containing a total of 25 mL of electrolyte was employed and thermostated using a water bath at 25.0 ± 0.3 °C.

Sensory analysis of the garlic samples was performed by Beacon Foods (Brecon, UK), who also supplied the garlic samples. Organoleptic testing was undertaken by mixing one gram of the raw garlic with 99 g of sour cream. A panel of ten untrained taste testers were then asked to rate the strength of the garlic/sour cream mix on a scale of zero to eight (where zero represented no flavor). From these results the mean and error of the mean were calculated.

3. Results and discussion

In the following work, first, the electrochemical detection of a model disulfide is evidenced (Section 3.1), followed by the use of real samples where the garlic organosulfur species are extracted using ethyl acetate (Section 3.2). Finally (Section 3.3), a strong correlation between the organoleptic and electrochemical methodologies is evidenced.

3.1. Propyl disulfide detection

Fig. 2 shows, the oxidation of 1.875 mM sodium bromide (electrolyte; 0.1 M $NaClO_4$ and 5% by volume ethyl acetate) recorded at a platinum macro-electrode, the voltammetric scan is started at 0.0 V (vs SCE) and swept anodically to +1.2 V, the electrode potential was subsequently scanned back to 0.0 V to complete the cyclic voltammogram (scan rate = 100 mV s^{-1}). On the forward sweep a large oxidative peak is observed at (0.97 V) corresponding to the one-electron oxidation of the bromide ions to bromine. On the reverse scan the corresponding reduction wave is observed at +0.88 V. The relatively small peak-to-peak separation (88 mV) for the system (as compared to previous results on carbon) is due to the use of a platinum electrode, where the interfacial electron transfer kinetics are significantly faster. It should be commented that at high bromide concentrations the electrochemical formation of bromine has been reported to be self-inhibiting with the associated formation of tribromide (Br_3^-) (Allen, Buzzeo, Villagrán, Hardacre, & Compton, 2005). This self-inhibition leads both to a minimisation of the forward wave and an associated decrease in the reductive peak height.

On addition of propyl disulfide to the solution the oxidative voltammetric wave is observed to increase. Conversely, the dashed